binant baculovirus-infected insect cells and recombinant adenovirus expressing rabies glycoprotein (rAdV). We also investigated immunity induced by those protein and virus in experimental mice.

Methods: For construction of rGP and rAdV, G gene from field isolate (SKRRD9901PJ) was altered to replace with the codons preferred in insect cell and mammalian cell for its high-level expression. In baculovirus expression system, baculovirus DNA including genes of chaperones such as heat shock proteins was used in order to prevent aggregation of rGP and elevate its solubility. The replication-defective adenovirus expressing rabies glycoprotein was created by homologous recombinant in HEK 293 cell using human adenovirus serotype 5 DNA deleted the early transcribed E1 and E3 genes. Five female ICR mice were immunized two times in a 30-day interval with 0.4 mg of insect cell lysate including rGP given intramuscularly. Groups of five female mice were immunized with ten-fold serially diluted rAdV (10^7—10^4 $TCID_{50}$) given intramuscularly and 10^8—10^6 $TCID_{50}$ titer given orally. Mice were periodically bled under anaesthesia by retro-orbital puncture. Virus-neutralizing antibodies (VNA) were determined with CVS-11 virus on BHK-21 cells as FAVNT method and commercial ELISA (Bio-Rad).

Results and conclusion: While the VNA titers by single inoculation of rGP were low and did not continue long in existence, titers by booster injection exceeded the 0.5 IU by 180 days. However, these immunities were inferior to those by commercial inactivated vaccines. All mice immunized intramuscularly with low titer of rAdV developed high VNA within 7—14 days after one inoculation. In experiment of oral immunization of rAdV, although titer of VNA varied in individual mice, geometric mean titers showed dose dependent. VNA titers of above 2.5 IU could be elicited after oral immunization by rAdV with titer of 10^6 $TCID_{50}$ and those antibodies were lasted by 180 days without decline of titers. As a conclusion, rAdV induced high titers of rabies VNA compared to rGP and was suitable as material to induce protective immunity against rabies.

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Antibiotics - Gram Positive (Poster Presentation)

44.001

The Antimicrobial Resistance of Streptococcus pneumonia by E-test Method

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Pneumococcus is among most common gram-positive bacteria causing infection in human. Unfortunately resistance of Pneumococcus is increasing daily like other bacteria. Considering that no useful research regarding rate of resistance of Pneumococcus against ceftriaxone has been done by E-test method (at least in Isfahan province), the above study can be a base for future studies about determination of increase or decrease in Pneumococcus resistance rate.

separated from clinical samples of patients presenting to Al-Zahra Hospital, and then MIC (Minimal Inhibitory Consentration) of antibiotics ceftriaxone and penicillin on the organisms was determined using E-Test method. Quality control was done using Pneumococcus ATCC 49619. After editing and entering in to computer, data were analyzed using SPSS-13 and WHONET-5.

Results: This study was performed on 98 patients with age range between 5 to 10 years. Among patients, 47% are female and 53% are male. The studied samples are 55% from throat, 20% from CSF, 16.5% from blood, 3% from pleural fluid, 3% from ear (patients with otitis), 1% (1 person) from abscess and 1% (1 person) from wound. Separated Pneumococci showed about 30% sensitivity to penicillin whereas MIC of ceftriaxone for cases other than meningitis is about 90% sensitivity and this MIC for meningitis considering lesser penetration of the drug in CNS, is 81.5% sensitivity.

Conclusion: Penicillin is not an effective drug for coverage of Pneumococcus even in children, but considering effectiveness of ceftriaxone, this drug alone is sufficient in cases suspicious of Pneumococcus at early presentation and vancomycin is not needed before culture result and antibiogram.

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44.002

Epidemiology of Pneumococcal Infection and Antimicrobial Resistance of S. pneumoniae Iso-Lates Gained from Adults with and Without Low Immune Status (Far East of Russia, 2003—2006)

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Despite of the gained success in diagnostics and treatment, pneumococcal infection is still remaining the leading cause if pneumonias, meningitis etc in different groups of population, including groups with different risk factors such as age, immunodeficiency, chronical somatic diseases. On data of the previous research projects of pneumococcal infections in our city, the incidence of pneumococcal pneumonias in adults over 18 years is 36 per 100 000 of population. The aim of our research was to study epidemiology of the pneumococcal pneumonias in patients with low immune status and without any clinical immune disturbances.

Methods: we studied 140 isolates of S.pneumoniae gained from patients with pneumococcal pneumonias at the age of 18—40 years, without any others somatic or immune complications (group 1), and 65 isolates gained from patients of 18—72 years with hematology diseases (myeloma, leucosis, etc); antimicrobial resistance was studied on NCCLS standards with disk-diffusion and microdiltion methods; there were performed serotyping and PFGE.

Results: there were revealed only (0/6, 15%) of strains resistant to penicillin (group 1 vs.group 2); 34,2%/56, 9% strains resistant to tetracycline, 15, 7%/24, 6% strains resistant to erythromycin, 15%/18,4% resistant to levofloxacin, 55, 7%/40, 3% resistant to co-trimoxazole and 11,4%/12, 3%

resistant to clindamycin. The most prevalent serotypes were serotypes 19F (20%), 23F(15, 7%) and 18C (12, 6%) among isolates from the 1 group and 6B(18,4%), 9B (16, 9%) and 14 (27, 6%) among isolates of the 2nd group. On performing PFGE there were revealed more diversity in strains of the 1st group then in strains gained in the 2nd group of patients with low immune status (17 genotypes vs. 6 genotypes).

Conclusion: The morbidity on pneumococcal pneumonia is still remaining underestimated problem in our area and requires careful epidemiology surveillance. There is tendency to forming of epidemic clone which could play role in appearance of nosocomial pneumococcal infections in patietns with hematology diseases.

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44.003

Genotypic Characterization of Methicillin-resistant *Staphylococcus aureus* from a Teaching Hospital

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Background: Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a serious cause of nosocomial infection in Malaysia. Sixty-six MRSA isolates recovered from patients from Universiti Malaya Medical Centre, KL over a 4-year period (2003—2007) were studied to determine the clonal relationship of sporadic (year 2004 and 2007) and outbreak (year 2003) isolates.

Methods: Polymerase chain reaction was carried out on the isolates to detect the *mec*A gene which is primarily responsible for the methicillin resistance in *Staphylococcus aureus*. Pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested chromosomal DNA and antimicrobial susceptibility tests were carried out.

Results: Antimicrobial susceptibility tests generated 34 antibiograms with 100% of isolates being susceptible to vancomycin. Majority of the isolates showed susceptibility towards fusidic acid (91%), clindamycin (82%), vancomycin (100%) and rifampin (89%), indicating that these antibiotics will be/are effective in treating MRSA infections. A dendrogram based on the clustering of the antibiograms showed two major clusters (AM1 and AM2), with AM 2 being primarily sporadic isolates. The majority of outbreak isolates were represented by 3 distinct antibiograms that clustered in AM1. Isolates susceptible to gentamicin, sulfamethoxazole-trimethoprim(SXT) and tetracycline gave distinctly different PFGE profiles, and were clustered together. Chi-square or Fishers exact test showed that there was a significant difference ($p < 0.05$) in antimicrobial resistant between sporadic and outbreak isolates for amikacin, SXT, tetracycline and gentamicin.

Fifty-nine isolates (89%) were *mec*A positive and showed the presence of the 533bp amplicon, while 7 isolates (11%) were *mec*A negative. These 7 isolates however showed phenotypic resistance to methicillin. Genotyping by PFGE showed 55 profiles, consisting of 13—17 bands. Among the 25 outbreak isolates, 22 PFGE profiles were obtained, suggesting that the outbreak did not originate from a single point source. Majority (93%) of isolates from year 2007 were clustered together. The diverse PFGE profiles obtained from the sporadic isolates indicated that MRSA were genetically diverse, and the diversity may be explained by the patients coming from different hospital wards.

Conclusion: The clonal relationship of outbreak and sporadic isolates and their antimicrobial susceptibility were determined. These findings aid in hospital infection control, lower the mortality rate of patients and reduce monetary loss to the hospital. The reemergence of isolates susceptible to amikacin- gentamicin-SXT-tetracycline may denote presence of MRSA in the community where such antibiotic exposure is minimal.

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44.004

'Click Chemistry' Synthesis of Macrolide Derivatives with Anti-MRSA and Anti-VRE Activity

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Although macrolides, including erythromycin A (EMA), have been widely prescribed for more than 50 years, the emergence of widespread bacterial resistance is a serious and expanding problem. There is a great medical need for new macrolide antibiotics to specifically cope with the problems of antibiotic resistance, especially to combat methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE) strains of bacteria. We have now re-examined various derivatives of EMA, previously synthesized at The Kitasato Institute, evaluating them against 12 types of Gram-positive bacteria, including macrolide-resistant strains, and one Gram-negative organism. We found that 11,12-di-O-butyryl-8,9-anhydroerythromycin A 6,9-hemiketal (EM413) showed moderate minimum inhibitory concentration (MIC) against four types of MRSA strains and two types of VRE strains. We subsequently investigated several 11,12-di-O-acyl-8,9-anhydroerythromycin A 6,9-hemiketal derivatives to elucidate their structure-activity relationships against anti-MRSA and VRE bacteria. After screening various diacyl compounds, 11,12-di-O-isobutyryl-8,9-anhydroerythromycin A 6,9-hemiketal (EM1015) was found to be active against MRSA and VRE strains. Furthermore, to obtain higher potency compounds, we synthesized new 8,9-anhydroerythromycin A 6,9-hemiketal derivatives using a copper catalyzed azide-alkyne cyclization reaction ('click chemistry'). Using click chemistry, we replaced the cladinose moiety on the C3 hydroxy with a -CH2-CCH group, to enable a fast SAR. This was done because the cladinose of EMA is not essential for its antibacterial activity, plus this moiety induces macrolide-drug-resistance. We then discovered that the propargyl and some triazole groups can be substituted, instead of cladinose, to produce better anti-MRSA and anti-VRE activity. One of 10 kinds of triazole compounds, the adamantyl triazole product (EM1035), was generated as a potentially